Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

By the above amendments, claim 15 is amended to recite features of original claim 16 and claims 16 and 23-46 are cancelled without prejudice. Claims 1-15 and 17-22 remain pending.

Over the past decade, numerous methods that combine the power of combinatorial chemistry and high-throughput screening have been developed to generate novel ligands that are critical to both basic research and therapeutic applications. Among these methods, the adaptive molecular evolution techniques with RNA, also known as SELEX, is particularly powerful thanks to the unique features of the RNA molecules, which not only carry information for their own replication but also fold into well-defined shapes. The novel ligands, or aptamers, generated by this SELEX process are capable of binding to a wide variety of targets with high affinity and specificity.

There has been a growing need in the drug discovery and functional genomics fields to develop methods that would yield aptamers against multiple targets in a single selection experiment. Experiments have been performed against multi-subunit enzymes, viral particles, organelles, and entire cells, with different degrees of success. In some cases, different families of ligands were identified for different targets in the mixture. However, these aptamers recognize the most abundant or easily recognizable target sites, which are not necessarily the most desired ones. No existing literature, i.e. none of the experiments performed so far and none of the currently available methods, offers an approach which is capable of generating different ligands to all of the targets in a mixture.

The most important step in the SELEX procedure is the partitioning step in which the target-binding species is physically separated from the non-binding species. The immobilization matrix used as partitioning device can act as an unwanted target to generate unwanted aptamers that often dominate the selected pool. Several methods are commonly in use to avoid this problem. First, matrix-binding species may be eliminated by negative selection against the matrix, i.e., collecting the fraction not bound to the matrix. However, in early cycles, when the copy number of each clone is low, this extra handling may increase the chance of stochastic events in which a particular sequence is lost. In later rounds, when the matrix bound species becomes the dominant sequence population in a pool, this method may not be efficient enough to eliminate them. Second, since the number of binding sites on the

surface of the matrix is fixed, increasing the amount of the target may change the ratio of the matrix-binding species to target-binding aptamers in favor of the recovery of the target-binding aptamers in the partitioning step. But the number of binding sites on the matrix can be extremely large compared to that on the target even at its highest level, thus rendering this method ineffective. In addition, higher target amounts will also favor the isolation of ligands with lower affinity, thus decreasing the efficiency of selection. Third, alternating use of different types of matrices should theoretically eliminate molecules binding to either. This method is less effective than might be expected because the difference between the commonly used matrices is not sufficient to discriminate against common aptamers that can bind via less specific hydrophobic interactions. Because of the lack of an efficient and specific negative selection method, the background problem is still a major reason of failure during *in vitro* selection experiments.

The present invention is directed to overcoming these deficiencies.

The rejection of claims 1, 2, 4, 7-11, and 14-16 under 35 U.S.C. § 102(b) as anticipated by U.S. Patent No. 5,792,613 to Schmidt et al. ("Schmidt") is respectfully traversed.

Schmidt teaches a method for selecting an RNA aptamer that binds a nucleic acid molecule by way of shape recognition. According to this method, a large RNA population is pretreated with a sufficient quantity of blocking oligodeoxynucleotide to preclude potential base pairing interactions between RNA molecules in the population and the selecting nucleic acid molecule. After pretreatment, the candidate RNA population is subjected to a selection process whereby it is first contacted with the selecting nucleic acid molecule to allow non-covalent binding of the RNA aptamer to the structural element of the selecting nucleic acid molecule. The resulting RNA aptamer:nucleic acid molecule complex is then separated from the remaining free RNA molecules, after which the complex is dissociated. The selected RNA population is thereby enriched for RNA aptamers that bind the selecting nucleic acid molecule by way of shape recognition. Successive rounds of selection are carried out until at least one characteristic sequence motif becomes apparent, that is, until one specific aptamer sequence or a family of aptamers dominates the pool of selected aptamers. After each round of selection, the selected RNA population, enriched for the RNA aptamer of interest, is preferably reverse transcribed to cDNA, amplified, then transcribed into RNA before beginning the next round of selection.

The presently claimed invention covers a process that begins where the modified SELEX process of Schmidt ends (i.e., with an enriched pool of RNA that contains a

predominant RNA aptamer species or family). Thus, Schmidt does not teach the first step as recited in claim 1, which covers "treating a first pool of RNA ligands that collectively bind more than one target under conditions effective to reduce the concentration or eliminate the presence *of one or more predominant target-binding RNA ligands* from the first pool of RNA ligands" (emphasis added). As a consequence, Schmidt cannot teach the subsequent steps, which recite "amplifying the RNA ligands in the treated first pool, thereby forming a second pool of RNA ligands that is enriched in one or more non-predominate target-binding RNA ligands of the first pool but not the one or more predominate target-binding RNA ligands thereof" and "identifying from the second pool one or more predominate target-binding RNA ligands that are present in the second pool at a higher concentration than other target-binding RNA ligands."

For these reasons, Schmidt clearly does not anticipate the method recited in claim 1, or claims 2-14 dependent thereon.

With respect to amended claim 15, this claim is directed to a method of reducing the concentration or eliminating the presence of *unwanted target-binding species* from a pool of RNA ligands. Schmidt fails to teach "isolating one or more unwanted target-binding RNA ligands [from a pool of RNA ligands that includes both wanted and unwanted target-binding RNA ligands]," "sequencing the one or more unwanted target-binding RNA ligands," and "treating the pool under conditions effective to reduce the concentration or eliminate the presence of the one or more unwanted target-binding RNA ligands from the pool of RNA ligands."

Nowhere does Schmidt teach or suggest isolating and sequencing one or more *unwanted* target-binding RNA ligands, let alone treating the pool to remove the *unwanted* target-binding RNA ligands. To the contrary, Schmidt is directed to isolating and sequencing, from a selected RNA population, an RNA aptamer of interest (col. 3, lines 28-32). Accordingly, Schmidt does not anticipate the method recited in amended claim 15.

In view of the foregoing, the rejection of claims 1, 2, 4, 7-11, and 14-16 under 35 U.S.C. § 102(b) as anticipated by Schmidt is improper and must be withdrawn.

The rejection of claims 1, 2, 4, 7-11, and 14-16 under 35 U.S.C. § 102(b) as anticipated by U.S. Patent No. 5,582,981 to Toole et al. ("Toole") is respectfully traversed.

Toole teaches a method to determine an oligonucleotide sequence which binds specifically to a target. This method is basically a modification of the SELEX procedure, whereby the oligomers (of the initial pool) contain portions that permit amplification. The process involves providing a mixture containing the above-described oligomers and

incubating the oligomer mixture with the target substance coupled to a support to form complexes between the target and oligomers bound specifically thereto. The unbound members of the oligonucleotide mixture are removed from the support environment and the complexed oligonucleotide(s) are recovered by uncoupling the target substance from the support. The recovered oligonucleotides are amplified, and the recovered and amplified oligonucleotide(s) (which had been complexed with the target) are sequenced.

The method of claim 1 of the present application is distinguishable from the modified SELEX procedure taught by Toole. As stated in the paragraph bridging pages 10-11 of the present application, in a SELEX experiment, genetic selection is applied directly to populations of RNA molecules that possess both genotypes (a sequence) and phenotypes (a binding activity that varies according to sequence). The conventional SELEX method attempts to recapitulate the natural Darwinian evolution process, in which the selection is based on phenotype (e.g. binding capability possessed by a folded RNA) and amplification is based on genotype (base paring during PCR). While the fitness of molecules (their ability to be enriched) may be also affected by their relative efficiency of enzyme-mediated replication, this is intentionally minimized, rather than explored, in the process of the experiment in order to keep the selection pressure solely on the phenotype. The nucleotide sequence of a nucleic acid molecule, as the physical embodiment of the information encoded therein, can be used in itself as the criterion for either positive or negative selection. This feature has been explored in some molecular computation experiments to solve hard combinatorial optimization problems. But when a sequence functions as the genotype of an organism, it is normally not accessible and subject to selection; and when it acts as the genotype of an aptamer in an ideal "single target selection," it is unknown until it is enriched according to its phenotype to the point of its identification. Once the sequence of an aptamer is identified in such an experiment, it loses its value of being a section criterion for itself, since by then the selection has achieved its practical goal and is considered finished. The present invention provides a scheme of negative selection according to genotype, which utilizes the sequence information to reduce the relative size of particular aptamer populations during the process of selection against multiple targets. More specifically, it allows the resumption of selection and amplification to identify less abundant aptamers to other targets, once an aptamer family is identified due to its high growth rate.

Thus, although Toole teaches separating non-aptamer sequences (that do not bind the target) from aptamer sequences (that bind the target), Toole fails to teach a negative selection step from the enriched aptamer pool. Specifically, Toole fails to teach the

"treating" step and the subsequent "amplifying" and "identifying" steps of claim 1, as well as the "isolating," "sequencing," and "treating" steps of claim 15. For this reason, Toole cannot be said to teach or suggest each and every limitation of claim 1 (or claims 2, 4, 7-11, and 14 dependent on claim 1) or amended claim 15.

Accordingly, the rejection of claims 1, 2, 4, 7-11, and 14-16 under 35 U.S.C. § 102(b) as anticipated by Toole is improper and must be withdrawn.

The rejection of claims 3, 5, 6, 12, 13, and 17-22 under 35 U.S.C. § 103(a) for obviousness over Schmidt in view of U.S. Patent No. 6,344,321 to Rabin et al. ("Rabin") is respectfully traversed.

The teaching and deficiencies of Schmidt are recited above.

Rabin is directed to methods for generating nucleic acid ligands to HGF and c-met, using the SELEX process for ligand generation. Figure 2 of Rabin illustrates RNaseH cleavage primers used in hybridization truncate SELEX. Basically, RNaseH cleavage primers are used simply to remove the known 5'- and 3'-terminal nucleic acid sequences from the randomized aptamer sequence selected during the SELEX procedure.

Even if one of ordinary skill in the art were to combine the teachings of Schmidt and Rabin, the combination would teach RNaseH treatment of wanted RNA ligands that were selected during SELEX. Unlike Rabin, the RNaseH treatment in accordance with the claimed invention is used to destroy the predominant aptamer species/family from a particular SELEX pool (i.e., an unwanted RNA aptamer). Thus, Rabin fails to both overcome the above-described deficiencies of Schmidt and teach the use of RNaseH as recited in claims 12, 13, 17, and 18.

Accordingly, the obviousness rejection of claims 3, 5, 6, 12, 13, and 17-22 is improper and must be withdrawn.

In view of all of the foregoing, it is submitted that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: September 6, 2006

/Edwin V. Merkel/
Edwin V. Merkel
Registration No. 40,087

NIXON PEABODY LLP
Clinton Square, P.O. Box 31051
Rochester, New York 14603-1051
Telephone: (585) 263-1128
Facsimile: (585) 263-1600